THE ROLE OF THE CRP/CAMP PROTEIN COMPLEX IN DNA TRANSCRIPTION

DNA molecules direct the synthesis of specific RNA and protein molecules. In the early stages of protein synthesis, specific regions of the DNA (genes) are copied into short strands of RNA that retain all of the genetic information of the DNA sequence from which they were copied. The process by which RNA molecules are synthesized from the coding regions of DNA is known as DNA transcription. The RNA polymerase enzyme, whose function is to make a RNA copy of a DNA sequence, catalyzes the synthesis of these RNA molecules. The amount of RNA made from a particular region of DNA is controlled by gene regulatory proteins that bind to specific sites on DNA close to the coding sequences of a gene. In this highlight we describe experiments addressing how a particular gene regulatory protein controls RNA transcription from DNA.

One useful model of such a protein is the cyclic AMP receptor protein (CRP) of *E. coli.* Upon binding cyclic adenosine monophosphate (cAMP), CRP undergoes a conformational change that, in turn, promotes binding to specific DNA sequences. The CRP/cAMP complex, upon binding DNA, produces a bend in the DNA that causes it to wrap around the RNA polymerase to promote DNA transcription.

A method well suited to directly study the structure of proteins and DNA in solution, where transcription takes place, is small-angle neutron scattering (SANS). The radius of gyration, R_g, which can be used to measure conformational changes, and the structure of the molecule in solution can be determined from an analysis of the scattered neutron intensity versus Q.

Particularly powerful is the contrast variation technique [1] in which isotopic substitution of D for H in the solvent is routinely used to change the scattering from a macromolecule without affecting its biochemistry. In the case of a two-component complex such as CRP/cAMP/DNA (cAMP is considered to be part of the CRP component), the neutron scattering length density of CRP is quite different from that of DNA. In this case, the scattered intensity at each Q value is expressed as the sum of three terms, each of which is the product of an unknown component intensity and a known contrast term. (The contrast is the difference between the scattering length density of a component and that of the solvent.) Thus, the scattering from the complex in solution can be separated into component intensities by measuring the scattered intensity of the complex, $I(Q)$, at a minimum of three contrasts obtained from different D_2O/H_2O buffer mixtures.

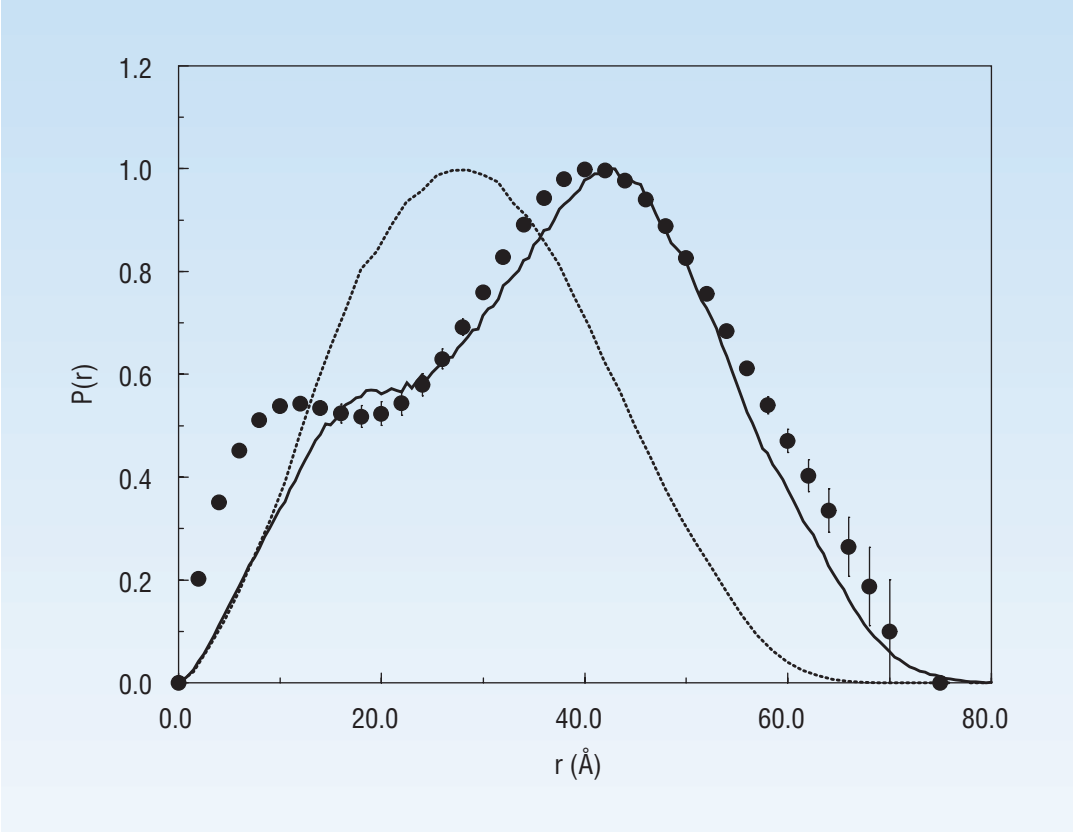

FIGURE 1. The distance distribution functions, P(r), of the CRP component of the CRP/cAMP/DNA complex obtained from the SANS data (●), the energy-minimized x-ray crystal structure (...) [5,6] and the energy-minimized model structure (—).

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Recent SANS measurements of CRP/DNA complexes [2] confirmed, in solution, the bending of the bound DNA that was observed in an early x-ray crystal structure of the complex [3]. However, the *R*g value for the complex was larger than that predicted from the same crystal structure. SANS confirmed experimentally that this value does not change with concentration. Thus, the increase in R_g is not due to aggregation, but it could result from an increase in the R_g of the CRP component upon DNA binding. Such a conformational change would be apparent in the SANS solution measurements: it was not evident in the crystal structure [3].

To obtain the scattered neutron intensity of the CRP component, a contrast variation series of SANS measurements was performed on CRP/DNA complexes in 0 %, 15 %, and 70 % D_2O/H_2O buffer solutions. The *R*g values were found to be the same, (28 Å to 30 Å), for all three cases. This clearly indicates that the CRP component is the main reason that R_{gCRP} was larger than originally expected. It was found from the *Q* behavior of the CRP component intensity that $R_{gCRP} = 28.5 \pm 0.3$ Å, which is ≈ 6 Å larger than the 21.6 ± 0.2 Å value observed in solution for CRP alone [4]. It is also ≈ 6 Å larger than the 22.6 Å value predicted for the CRP component from an energy-minimized x-ray crystal structure of the complex by Parkinson *et al*. [5], with cAMP incorporated as in Passner and Steitz [6].

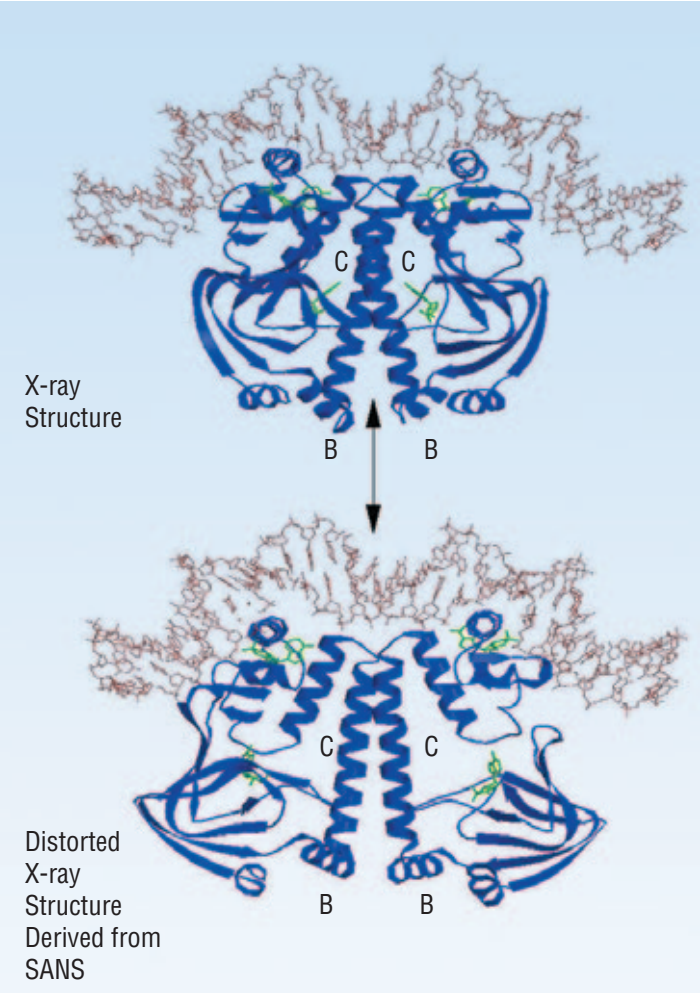

FIGURE 2. The top molecule is the energy-minimized x-ray crystal structure [5,6] of the CRP(blue)/cAMP(green)/DNA(brown) complex. The bottom molecule is the energy-minimized model structure which fits the SANS data.

To model the solution structure of the CRP/DNA complex, the energy-minimized x-ray structure [5,6] was distorted in the regions thought most likely responsible for the conformational change in CRP upon DNA binding [7]. The distance distribution function, *P*(*r*), was calculated [4] from the energy-minimized distorted conformation and compared to that obtained from the SANS data. As shown in Fig. 1, the *P*(*r*) function calculated from the model structure clearly fits the experimental data better than that from the x-ray crystal structure [5,6]. A molecular representation of the energy-minimized x-ray crystal structure [5,6] is shown in Fig. 2, along with the model structure that fits the SANS data.

The experimentally observed conformational change in CRP upon DNA binding may play a role in the enhancement of transcription of DNA by CRP. Perhaps this occurs through its contacts with RNA polymerase that is bound on the DNA at a site adjacent to the CRP binding site. This is the subject of further ongoing SANS studies.

REFERENCES

[1] H. B. Stuhrmann and A. J. Miller, Appl. Cryst. **11**, 325 (1978).

[2] Y. Shi, S. Wang, S. Krueger, and F. P. Schwarz, J. Biol. Chem. **274**, 6946 (1999).

[3] D. B. McKay, and T. A. Steitz, Nature **290**, 744 (1981).

[4] S. Krueger, I. Gorshkova, J. Brown, J. Hoskins, K. H. McKenney, and F. P. Schwarz, J. Biol. Chem. **273**, 20001 (1998).

[5] G. Parkinson, C. Wilson, A. Gunasekera, Y. W. Ebright, R. E. Ebright, and H. M. Berman, J. Mol. Biol. **260**, 395 (1996).

[6] J. M. Passner and T. A. Steitz, Proc. Natl. Acad. Sci. USA **94**, 2843 (1997).

[7] N. Baichoo and T. Heyduk, J. Mol. Biol. **290**, 37 (1999).